



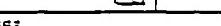
LADOLLA CANCER RESEARCH FOUNDATION

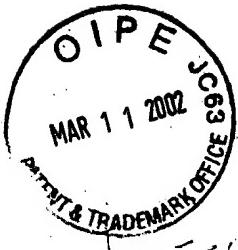
ANIMAL USAGE FORM

AUF 141-3

1. PRINCIPAL INVESTIGATOR WAYNE A. BORDER, M.D.	OFFICE PHONE 226	HOME/EMERGENCY PHONE (714) 770-4602			
2. OTHER INVESTIGATOR LUCIA LANCINO, PH.D.	220	519-0609			
3. SENIOR TECHNICIAN 					
4. PROJECT TITLE ANTI-HUMAN TGFβ CYCLIZED PEPTIDE					
5. GRANT NUMBER, IF ANY 250200	NEW <input checked="" type="checkbox"/>	RENEWAL <input type="checkbox"/>	PILOT <input type="checkbox"/>	PROJECT NUMBER 	
6. START DATE 	END DATE 	MICE <input type="checkbox"/>	RATS <input type="checkbox"/>	RABBITS <input checked="" type="checkbox"/> 2	OTHER (SPECIES)
7. PROJECT GOAL (SEE INSTRUCTIONS) To produce quantities of anti-human TGF β cyclized peptide for use in kidney disease research.					
8. RATIONALE (SEE INSTRUCTIONS) Rabbits produce high quality antiserum which can be used for identification of human TGF β in tissue samples and in vitro assays to study progression of kidney injury.					
9. DESCRIBE USE OF ANIMALS (SEE INSTRUCTIONS) All injections/bleedings to be performed by animal care facility personnel. <ol style="list-style-type: none"> 1. Pre-bleeding 20 ml from ear vein. 2. Inject 50μg TGFβ cyclized purified peptide (0.5 ml antigen in PBS + 0.5 ml FCA) subcutaneously in 2 sites. 3. After one month, boost with 125 μg antigen (0.25 ml antigen in PBS + 0.25 ml incomplete adjuvant) subcutaneously, 2 sites. 4. After 10 days, bleed 50 ml from alternating ear veins 3 times. 5. Repeat steps 3-4 at 4-6 week intervals. 					

CONFIDENTIAL

11. SUTHERLAND (SEE INSTRUCTIONS)			
DURING PROJECT	<input type="checkbox"/>	METHOD OR TECHNIQUE	CQ.
END OF PROJECT	<input checked="" type="checkbox"/>	G.Q.	CERV. DISLOC.
			OTHER SPECIMEN
RETAIN CARCASSES? YES <input type="checkbox"/>			
FOR RE <input type="checkbox"/> NO <input checked="" type="checkbox"/>			
12. SIGNATURES			
1) 	DATE / /	U2 05334	DATE / /
2) 	DATE / /		



Injection of 2 rabbits with Linear FP-103 peptide
no 86-82 from TGF β .

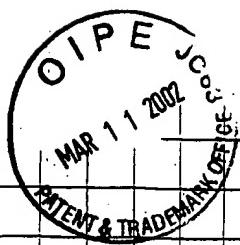
2 " with Glycylated FP-103 " .
no 88-83

~~coupling~~

Procedure: 2 mg / rabbit of each peptide.

- 2 mg peptide were dissolved in 250 μ l DDM
(add to the solution to couple the peptide)
- 0.5 mg Methylated BSA was ~~mixed~~ ~~coupled~~
~~coupling agent~~
(Sigma A1009 : H₂BSA)
- volume 3 ml
- Added 250 μ l Freud's adjuvant complete.
- mixed 1 h with homogenizer.
- 0.5 ml solution was injected in each rabbit.

Note: Both peptides were difficult to dissolve.
they were not fully purified.



Injection of 2 ml with linear TGF β peptide

Celite

PG peptide

Procedure

except Friend's incomplete adjuvant was used

For mice: KLN-peptides were already coupled

It only needs F i d s m i n i 14 C C

object 0.5 ml

Prob 7, m^o 1286 for PG = A1, F 1:85
1285